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Pea *VEGETATIVE2* Is an *FD* Homolog That Is Essential for Flowering and Compound Inflorescence Development

Frances C. Sussmilch,[a] Ana Berbel,[b] Valérie Hecht,[a] Jacqueline K. Vander Schoor,[a] Cristina Ferrándiz,[b] Francisco Madueño,[b] and James L. Weller[a,1]

[a] School of Biological Sciences, University of Tasmania, Hobart, Tasmania 7001, Australia
[b] Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-Universidad Politécnica de Valencia, Valencia 46022, Spain

ORCID IDs: 0000-0002-2460-1068 (C.F.); 0000-0003-2423-8286 (J.L.W.)

As knowledge of the gene networks regulating inflorescence development in *Arabidopsis thaliana* improves, the current challenge is to characterize this system in different groups of crop species with different inflorescence architecture. Pea (*Pisum sativum*) has served as a model for development of the compound raceme, characteristic of many legume species, and in this study, we characterize the pea *VEGETATIVE2* (*VEG2*) locus, showing that it is critical for regulation of flowering and inflorescence development and identifying it as a homolog of the bZIP transcription factor *FD*. Through detailed phenotypic characterizations of *veg2* mutants, expression analyses, and the use of protein-protein interaction assays, we find that *VEG2* has important roles during each stage of development of the pea compound inflorescence. Our results suggest that VEG2 acts in conjunction with multiple FLOWERING LOCUS T (FT) proteins to regulate expression of downstream target genes, including *TERMINAL FLOWER1*, *LEAFY*, and MADS box homologs, and to facilitate cross-regulation within the *FT* gene family. These findings further extend our understanding of the mechanisms underlying compound inflorescence development in pea and may have wider implications for future manipulation of inflorescence architecture in related legume crop species.

INTRODUCTION

Inflorescences are the shoot structures that bear flowers, and their form and arrangement have important implications for reproductive success and ease of harvest in agricultural systems (Wyatt, 1982). Angiosperm species exhibit incredible diversity in inflorescence form, which derives from complexity and pattern of branching, the number and position of flowers, and the capacity of the inflorescence for continued growth (Weberling, 1992). At the tissue level, this variation can be attributed to differences in the identity and activity of the shoot meristems that produce each component of the inflorescence. Among many genes that have a role in the regulation of flowering, a subset also have a role in specifying the identity of vegetative, inflorescence, or floral meristems, and it is the interaction of these genes that determines how the inflorescence develops. Understanding the genes and regulatory interactions that underlie the development of different inflorescence forms is a crucial step to enable future optimization of inflorescence architecture for maximal crop productivity.

Like most plant processes, inflorescence development is best understood in the model species *Arabidopsis thaliana*. The Arabidopsis inflorescence is a simple raceme, in which flowers are borne directly on the main stem and the shoot apex remains indeterminate, with organogenesis balanced by self-renewal (Figure 1A). Two key genes, *FLOWERING LOCUS T* (*FT*) and *TERMINAL FLOWER1* (*TFL1*), have a major role in generating this inflorescence form, through antagonistic effects on the expression of meristem identity genes (Kardailsky et al., 1999; Kobayashi et al., 1999; McGarry and Ayre, 2012; Jaeger et al., 2013). FT and TFL1 both belong to the phosphatidylethanolamine binding protein family and individually interact with the basic leucine zipper (bZIP) transcription factors FD and FD PARALOG (FDP) to regulate expression of floral target genes within the apex (Abe et al., 2005; Wigge et al., 2005; Hanano and Goto, 2011). TFL1/FD complexes delay flowering and prevent upregulation of floral genes within the shoot apical meristem (SAM) to maintain shoot indeterminacy (Hanano and Goto, 2011). FT/FD complexes promote expression of floral genes, ultimately resulting in the induction of the MADS box gene *APETALA1* (*AP1*) in axillary meristems to specify floral identity (Abe et al., 2005; Wigge et al., 2005). *AP1* is also upregulated by the floral integrator and floral identity gene *LEAFY* (*LFY*), which acts independently of the *FT*/*FD* pathway (Ruiz-García et al., 1997; Abe et al., 2005; Wigge et al., 2005). Within floral meristems, *AP1* and *LFY* directly repress *TFL1* expression to maintain determinacy (Wagner et al., 1999; Kaufmann et al., 2010).

In this study, we investigate genes regulating development of the compound raceme of pea (*Pisum sativum*). Pea is an important crop plant and is also representative of other agronomically significant legume species within the Papilionoideae, including lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), common bean (*Phaseolus vulgaris*), and soybean (*Glycine max*), which share similar inflorescence architecture. Relative to the simple raceme of Arabidopsis, the compound raceme of pea has an additional level of branching, such that flowers are not directly borne on the main inflorescence stem but are instead borne on modified lateral branches, termed secondary inflorescences (I_2s; Figure 1B). Pea inflorescence development can thus be considered to consist of three distinct stages. During vegetative growth, the SAM has

[1] Address correspondence to jim.weller@utas.edu.au.
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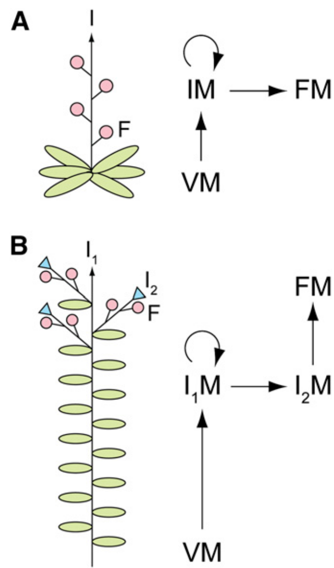

Figure 1. Inflorescence Development in Arabidopsis and Pea.

(A) The simple raceme of Arabidopsis.

(B) The compound raceme of pea.

For each species, a diagram of inflorescence architecture (left) and schematic of the meristem transitions involved in inflorescence development (right) are shown. In diagrams, arrows indicate indeterminate growth of the inflorescence stem (I; I_1), green ovals are leaves, pink circles are flowers (F), and blue triangles are stubs terminating each secondary inflorescence (I_2) axis. In schematics, straight arrows indicate meristem transitions and products, and circular arrows indicate meristem indeterminacy. Meristem abbreviations are as follows: vegetative meristem (VM), inflorescence meristem (IM), primary inflorescence meristem (I_1M), secondary inflorescence meristem (I_2M), and floral meristem (FM).

vegetative (V) identity and produces the main stem, bearing alternate leaves with vegetative axillary buds, which normally remain dormant. On commitment to flowering, the pea vegetative SAM undergoes a transition to a primary inflorescence (I_1) meristem (Ferguson et al., 1991), which we refer to here as the V/I_1 transition, the first stage of inflorescence development. The I_1 meristem is similar to the vegetative SAM, in that it remains indeterminate and produces the shoot tissues (stem and leaves) of the main stem. However, the I_1 is distinguished by the fact that it bears an axillary I_2 at each stem node instead of a vegetative bud (Singer et al., 1999). Specification of I_2 meristem identity is the second stage of pea inflorescence development. Each I_2 is leafless and determinate, terminating in a hairy stub after bearing several axillary flowers (Hole and Hardwick, 1976). Specification of floral meristem identity is the third and final stage of pea inflorescence development.

A number of key genes that regulate inflorescence development in pea have been identified based on characterization of pea mutants with altered inflorescence form (summarized in Supplemental Figure 1). Mutants of *PROLIFERATING INFLORESCENCE MERISTEM* (*PIM*), an *AP1* homolog, fail to correctly specify floral meristems (Taylor et al., 2002). In accordance with a conserved role as a floral meristem identity gene, expression of *PIM* is limited to floral meristems (Berbel et al., 2001). *DETERMINATE* (*DET*), a *TFL1* homolog (*TFL1a*), is expressed within the I_1 meristem, where it promotes SAM indeterminacy (Foucher et al., 2003; Berbel et al., 2012). *det* mutants exhibit conversion of the I_1 to an I_2, which terminates the main stem (Singer et al., 1990). Unlike Arabidopsis *TFL1*, *DET* has no influence on flowering time in pea, and this role is instead played by *LATE FLOWERING* (*LF*), a second pea *TFL1* homolog (*TFL1c*), which acts to delay flowering (Foucher et al., 2003).

Three other pea loci of particular interest for understanding the regulation of inflorescence development are *GIGAS*, *VEGETATIVE1* (*VEG1*), and *VEG2*. Plants carrying severe mutant alleles for any of these loci exhibit normal vegetative development but fail to develop I_2 or floral structures under long-day (LD) photoperiods, suggesting that these loci have critical roles in pea inflorescence development (Reid and Murfet, 1984; Murfet and Reid, 1993; Beveridge and Murfet, 1996). *GIGAS* has been characterized as the *FT* homolog, *FTa1*, which is thought to encode a graft-transmissible floral stimulus that can travel from leaf to apex to promote flowering (Beveridge and Murfet, 1996; Hecht et al., 2011), similar to Arabidopsis *FT* (Corbesier et al., 2007; Mathieu et al., 2007). The most severe *gigas* mutant, *gigas-2*, is nonflowering under LD only and is late-flowering with normal I_2 and floral morphology under short-day (SD) conditions (Murfet, 1992; Taylor and Murfet, 1994; Hecht et al., 2011). *VEG1* has been identified as *FULc*, a MADS box gene from the *AGAMOUS-LIKE79* (*AGL79*) clade, which appears to have a role in legume compound inflorescence development as a critical I_2 identity gene (Berbel et al., 2012). The SAM of the *veg1* mutant undergoes the V/I_1 transition and acquires I_1 identity at the same time as the wild type, but fails to subsequently specify I_2 meristems (Berbel et al., 2012). The *VEG2* locus has received the least attention of these three pea loci and has not been described in detail. *VEG2* is represented by two recessive mutant alleles, *veg2-1* and *veg2-2*, both generated by fast neutron mutagenesis (Murfet, 1992; Murfet and Reid, 1993).

Here, we characterize the roles of the *VEG2* locus during each stage of inflorescence development in pea through examination of the two *veg2* mutant alleles. We identify *VEG2* as a pea homolog of *FD* (*FDa*) and further investigate the possible mechanisms of *VEG2* function. Our findings reveal that *VEG2* plays a central role in the regulation of meristem identity, acting in conjunction with multiple FT proteins to regulate expression of *FT*, *TFL1*, and *LFY* homologs and key MADS box genes, *VEG1* and *PIM*, throughout development of the pea compound inflorescence.

RESULTS

VEG2 Acts across All Stages of Inflorescence Development

We first examined *veg2-1* and *veg2-2* mutant phenotypes to investigate the role(s) of *VEG2* during compound inflorescence development. Under both LD and SD photoperiods, the *veg2-1* mutant remained nonflowering throughout development and the weaker *veg2-2* mutant flowered later than wild-type (Figures 2A and 2B). A conspicuous feature of both *veg2* mutants was increased aerial branching, with lateral branches occupying aerial nodes in *veg2-1*, and the aerial nodes prior to the first flowering node in *veg2-2* (Figures 2A and 2C; Supplemental Figure 2). This may be linked to

the absence of flowers and pods in *veg2-1* and their delayed appearance in *veg2-2*, as increased branching is also observed in wild-type plants when flowers/pods are removed (Figure 2C; Supplemental Figure 2; Lockhart and Gottschall, 1961) and in other nonflowering mutants, *veg1* and *gigas* (Gottschalk, 1979; Beveridge and Murfet, 1996).

The fact that both *veg2* mutations impair the initiation of flowering suggested that they might affect the V/I_1 transition (Figure 1B). I_1 meristems are characterized by the expression of the *TFL1* homolog *DET*, and *DET* expression has been used as a developmental marker for I_1 meristem identity (Berbel et al., 2012). In nonflowering *veg1* and *gigas* mutants, the timing of *DET* induction is similar to the wild type, indicating that the V/I_1 transition is not affected under LD in these mutants (Hecht et al., 2011; Berbel et al., 2012; Supplemental Figure 3). We first examined *DET* expression in the late-flowering *veg2-2* mutant at weekly

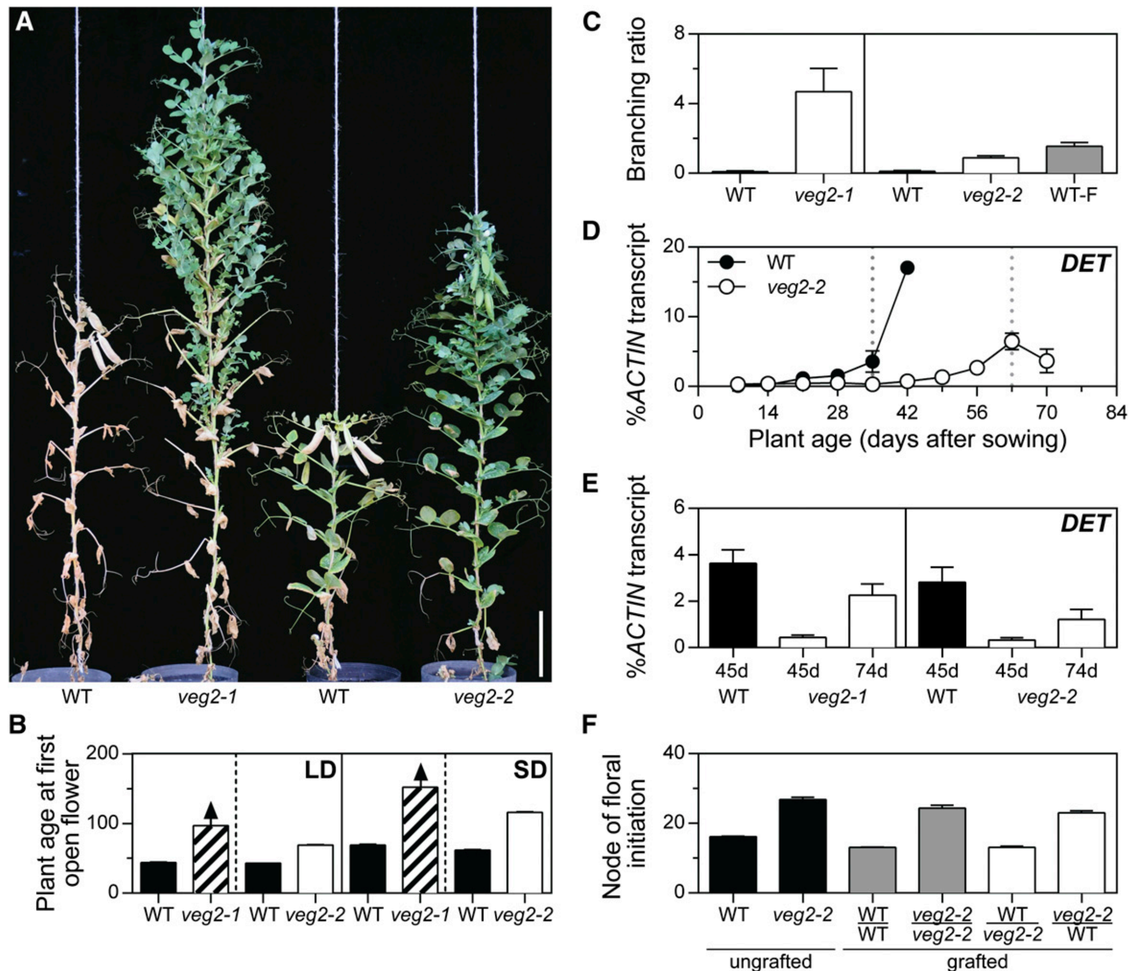

Figure 2. *VEG2* Acts Locally in the Apex to Promote Flower Initiation and Inflorescence Development.

(A) Representative *veg2-1* and *veg2-2* plants and their associated wild-type lines (wild-type siblings of *veg2-1* and NGB5839, respectively). Plants are shown 97 d after sowing in LD (18 light/6 dark). Bar = 10 cm.

(B) Plant age at first open flower (days after sowing) in LD (24 light) and SD (8 light/16 dark). Values represent mean ± SE for *n* = 3 to 6 plants. For nonflowering *veg2-1* mutants, bars with diagonal hatching and arrow show plant age at the end of the experiment.

(C) Ratio of total branch length to main stem length in intact *veg2* mutants, associated wild-type lines, and deflowered wild-type plants (WT-F, line NGB5839, each flower removed after anthesis). Mean values ± SE are shown for *n* = 5 to 6 plants grown in LD (24 light).

(D) and **(E)** Relative expression of *DET* transcript as a marker of primary inflorescence (I_1) identity in dissected shoot apices at weekly time points throughout development in *veg2-2* **(D)** and specific time points in both *veg2* mutants **(E)**. In **(D)**, developing floral buds were first macroscopically visible in the wild type 35 d after sowing and in *veg2-2* 63 d after sowing (broken lines) in LD (24 light). In **(E)**, time points correspond to early flowering stages in the wild type 45 d after sowing (45d) and *veg2-2* plants 74 d after sowing (74d) in LD (18 light/6 dark). Mean values ± SE are shown for *n* = 2 to 3 biological replicates.

(F) Node of floral initiation for graft combinations of the wild type (NGB5839) and *veg2-2* created by grafting 7-d-old scions onto 3-week-old stocks, controls comprising grafted plants with stock and scion of identical genotype, and ungrafted control plants. For each graft combination, the genotypes of scion (top) and stock (bottom) are shown, separated by a horizontal line. Values represent mean ± SE for *n* = 5 to 11 plants in LD (18 light/6 dark).

time points during development from seedling to flowering adult plant. *DET* induction was delayed by 3 to 4 weeks in *veg2-2* relative to the wild type, comparable to the ~4-week delay in the appearance of floral buds (Figure 2D). In a second experiment, we also examined *DET* expression in the more severe allele, *veg2-1*, but focused on only two time points, in view of limited availability of this sterile genotype. Time points were selected to coincide with the expected peaks in *DET* expression in the wild type and *veg2-2*. At 45 d after sowing, when the presence of floral buds indicated that the V/I_1 transition had occurred in the wild type, *DET* expression was 8- to 9-fold lower in the *veg2* mutants than respective wild-type plants (Figure 2E). By day 74, when floral buds were first visible in *veg2-2* apices and wild-type plants had senesced, *DET* expression had increased 4- to 5-fold in the *veg2* mutants. This suggests that *DET* induction is also delayed in *veg2-1*, similar to *veg2-2*, and that the V/I_1 transition is therefore delayed in both mutants. In addition, the fact that *DET* is eventually expressed in the nonflowering *veg2-1* mutant indicates that this mutant does acquire I_1 identity, but as no I_2 structures subsequently develop, we conclude that the next stage of inflorescence development, I_2 meristem specification, must be blocked in this mutant.

Next, we used grafting to investigate whether *VEG2* may contribute to the generation of a long-distance flowering signal, as is the case for the *GIGAS/FTa1* gene (Beveridge and Murfet, 1996; Hecht et al., 2011). Figure 2F shows that *veg2-2* scions grafted onto wild-type stocks flowered as late as self-grafted *veg2-2* control plants ($P = 0.729$), while wild-type scions grafted to *veg2-2* stocks flowered at a similar time to self-grafted wild-type plants ($P = 1.000$). These results indicate that *VEG2* cannot influence flowering across a graft union and suggest that *VEG2* instead acts locally within the shoot apex.

We next examined the weaker *veg2-2* mutant phenotype in more detail for insight into the role(s) of *VEG2* during the later stages of flowering, I_2 and floral development, which do not occur in the nonflowering *veg2-1* mutant. In the *veg2-2* mutant, I_2 morphology was abnormal at all reproductive nodes. Abnormal I_2 structures resembled the wild type in that they bore one or more axillary flowers, but unlike wild-type I_2 structures, which terminate in a stub, these had a bract subtending each flower and retained an indeterminate apex (Figures 3A to 3J). After producing one or two flowers, subsequent nodes of the *veg2-2* I_2 bore full compound leaves with vegetative axillary buds or axillary tertiary inflorescence (I_3) structures that reiterate the same abnormal I_2 pattern (Figures 3I and 3J). This phenotype suggests that I_2 identity is initially partly specified in *veg2-2*, but this identity is not maintained, and reversion to I_1 identity occurs. Consistent with this interpretation, *DET* was expressed in the indeterminate apex of the *veg2-2* I_2 at a similar level to that in the *veg2-2* I_1 apex, whereas in the wild type, expression of *DET* was limited to the I_1, and levels in the I_2 stub were negligible (Figure 3K). This indicates that *VEG2* has an important role not only in specifying but also in maintaining I_2 identity.

We also observed that flowers produced on *veg2-2* I_2 structures were frequently abnormal. Defects were most common in outer (sepal and petal) whorls (Figure 3L) and included fusion of floral organs to leaf or other floral tissue, a reduction in organ number, and organ displacement or malformation (Supplemental Figure 4). The severity of these floral defects decreased acropetally on the main stem axis, and flowers on I_2 structures at higher reproductive nodes showed normal morphology (Figure 3M; Supplemental Figure 4). These observations indicate a further role for *VEG2* in specification of floral meristems.

Previous study has shown that *PIM*, a pea *AP1* homolog, has a major role in specification of floral meristems in pea (Taylor et al., 2002). To determine whether *VEG2* could affect floral phenotype in the absence of functional *PIM*, we also examined the phenotype of the *pim-2 veg2-2* double mutant. *pim-2* mutant plants produce I_2 structures but fail to specify floral meristems correctly, and single flowers are typically replaced by groups of abnormal flowers (Taylor et al., 2002; Supplemental Figure 5). By contrast, no discrete units recognizable as flowers were observed in the *pim veg2-2* double mutant. Although structures with floral identity did form, these were limited to isolated floral organs that were fused to, or borne in the axils of, leaves or bracts on upper nodes of the main stem or branches (Supplemental Figure 5). This more severe phenotype of *pim veg2-2* relative to *pim* indicates that *VEG2* acts at least in part through genes other than *PIM* to specify floral meristem identity.

The *VEG2* Locus Corresponds to an *FD* Gene

Preliminary mapping indicated that *VEG2* was located toward the base of pea LGI (Murfet and McKay, 2012). We exploited the close synteny between pea and *Medicago truncatula* (Duarte et al., 2014) to search for an appropriate candidate for the *VEG2* locus. In the syntenic region of *M. truncatula* chromosome 5, we identified a homolog of the Arabidopsis bZIP transcription factor *FD* (Supplemental Figure 6). Mutants for *FD* genes in other species typically exhibit delayed flowering time (Abe et al., 2005; Wigge et al., 2005; Muszynski et al., 2006; Park et al., 2014), which is one of the features of the *veg2-2* mutant (Figure 2B). Isolation and mapping of the full-length coding sequence for the pea ortholog of this gene confirmed its location close to the *VEG2* locus (Supplemental Figure 6 and Supplemental Table 1).

With this gene as a likely candidate for *VEG2*, we investigated the legume *FD* family further. We identified *FD* genes from *M. truncatula*, soybean, common bean, and *Lotus japonicus*, excluding homologs of the closely related *AREB3/DPBF4* genes and other Group A bZIP transcription factors (Corrêa et al., 2008). Figure 4A shows that this approach identified three subclades of legume *FD* genes, which we have designated as *FDa*, *FDb*, and *FDc*. The *VEG2* candidate was included within the legume *FDa* subclade, which showed the greatest similarity to Arabidopsis *FD* and *FDP*. *FDa* and *FDb* subclades were represented in species from both galegoid and phaseoloid legume clades (Figure 4A) but were not apparent in other rosid I orders, including Rosales, Cucurbitales, and Malpighiales (Supplemental Figure 7 and Supplemental Table 2). This suggests that *FDa* and *FDb* subclades resulted from a legume-specific duplication event prior to the divergence of galegoid and phaseoloid lineages, ~54 million years ago (Lavin et al., 2005). Although *FDb* is present in *M. truncatula*, BLAST searches of pea transcript databases (Franssen et al., 2011; Kaur et al., 2012) and PCR approaches using degenerate and Mt *FDb*-specific primers (Supplemental Table 3) provided no evidence for an *FDb* ortholog in pea. *FDc* genes were identified only in the phaseoloid species soybean and common bean (Figure 4A). The gene present immediately upstream of *FD* in Arabidopsis, *LA RELATED PROTEIN 1C* (*LARP1C*), was found to be conserved close to all legume

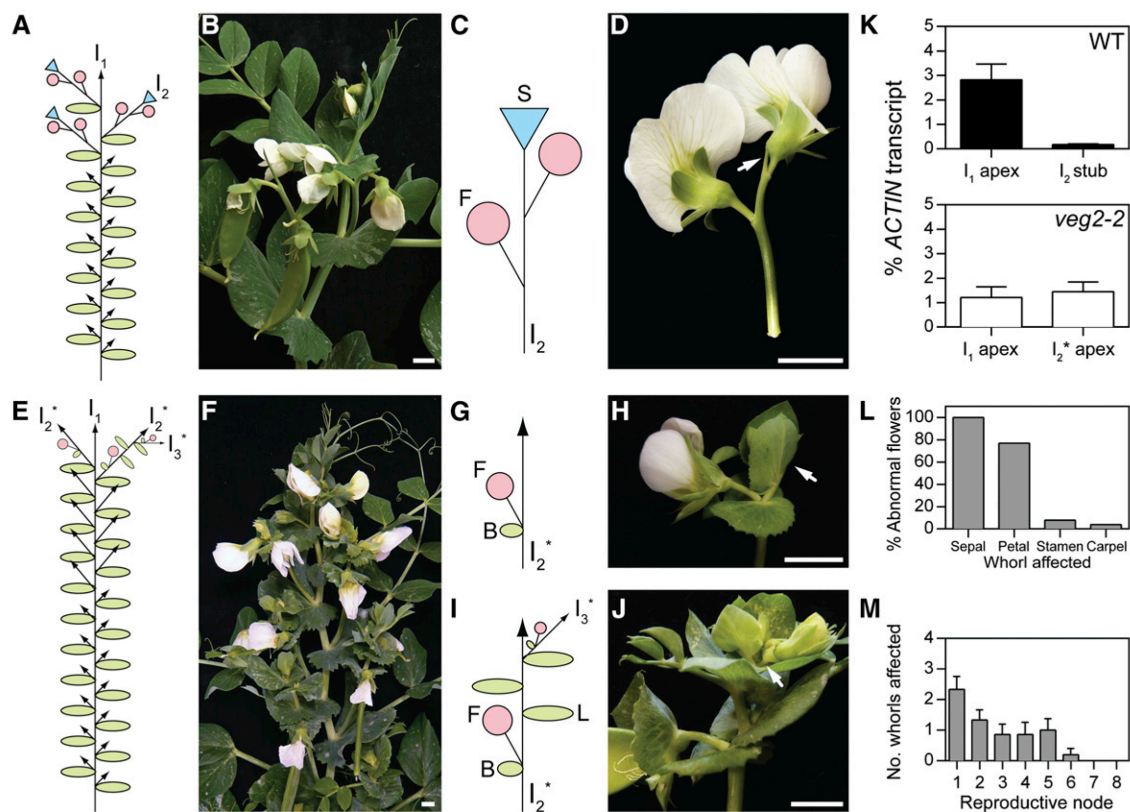

Figure 3. Inflorescence and Floral Morphology Is Abnormal in the *veg2-2* Mutant.

(A) to **(D)** Wild-type inflorescence structure in pea line NGB5839.

(A) Diagram of plant architecture.

(B) Photograph of reproductive nodes on the main stem.

(C) and **(D)** Diagram **(C)** and photograph **(D)** of the secondary inflorescence (I_2) which bears axillary flowers (F) and terminates in a stub (S; arrow).

(E) to **(J)** Inflorescence structure in *veg2-2*.

(E) Diagram of plant architecture.

(F) Photograph of reproductive nodes on the main stem.

(G) and **(H)** Diagram **(G)** and photograph **(H)** of a typical *veg2-2* I_2, which bears an axillary flower with subtending bract (B) and retains an indeterminate apex (arrow).

(I) and **(J)** Diagram **(I)** and photograph **(J)** of an older *veg2-2* I_2, which has a pod and subtending bract at the first node, three nodes with full compound leaves (L), and a flower on an axillary tertiary inflorescence (I_3; arrow in photograph).

Note all *veg2-2* I_2 structures are indeterminate, similar to **(H)**, and may develop additional nodes after bearing axillary flowers, similar to **(J)**.

(K) Relative expression of *DET* transcript as an indicator of primary inflorescence (I_1) identity in the dissected shoot apex during early flowering stages (I_1 apex; 45 and 74 d after sowing in wild-type NGB5839 and *veg2-2*, respectively) and in the I_2 (wild-type I_2 stub 59 d after sowing and *veg2-2* I_2 apex 74 d after sowing) in LD (18 light/6 dark). Mean values ± SE are shown for n = 2 to 3 biological replicates.

(L) Proportion of abnormal flowers in *veg2-2* defective in each of the four floral whorls for n = 26 flowers grown in LD (24 light).

(M) Number of whorls affected by floral defects at each reproductive node on *veg2-2* plants. Values represent mean ± SE for n = 7 plants grown in LD (24 light).

In diagrams, arrows indicate the potential for indeterminate growth, circles are flowers, triangles are terminal stubs, ovals are leaves or bracts, and asterisks indicate abnormal nature of structures. In photographs, bars = 1 cm.

FD genes investigated (Figure 4B; Supplemental Figure 8 and Supplemental Table 4). This microsynteny between genomic regions surrounding *FD* in Arabidopsis and legume homologs supports the probable common origin of Arabidopsis *FD* and all three legume *FD* subclades. No microsynteny was apparent between regions containing *FDP* in Arabidopsis and any legume *FD* genes.

Sequencing of *FDa* in the *veg2-2* mutant revealed a single nucleotide polymorphism (SNP) (G536A) directing a substitution (R179H) within the DNA binding, basic region of the bZIP domain (Figure 4C). This SNP cosegregated perfectly with *veg2-2* phenotype in a population of 114 F2 progeny that included 34 *veg2-2* mutants (Supplemental Figure 6). An arginine is highly conserved at this position in FD proteins from diverse angiosperm species and in 95% of all Arabidopsis bZIP family proteins (Supplemental Figure 9), which comprise 13 divergent groups with minimal sequence similarity outside of the bZIP domain (Corrêa et al., 2008). This high level of conservation strongly implies that this residue is important for general bZIP transcription factor function. The

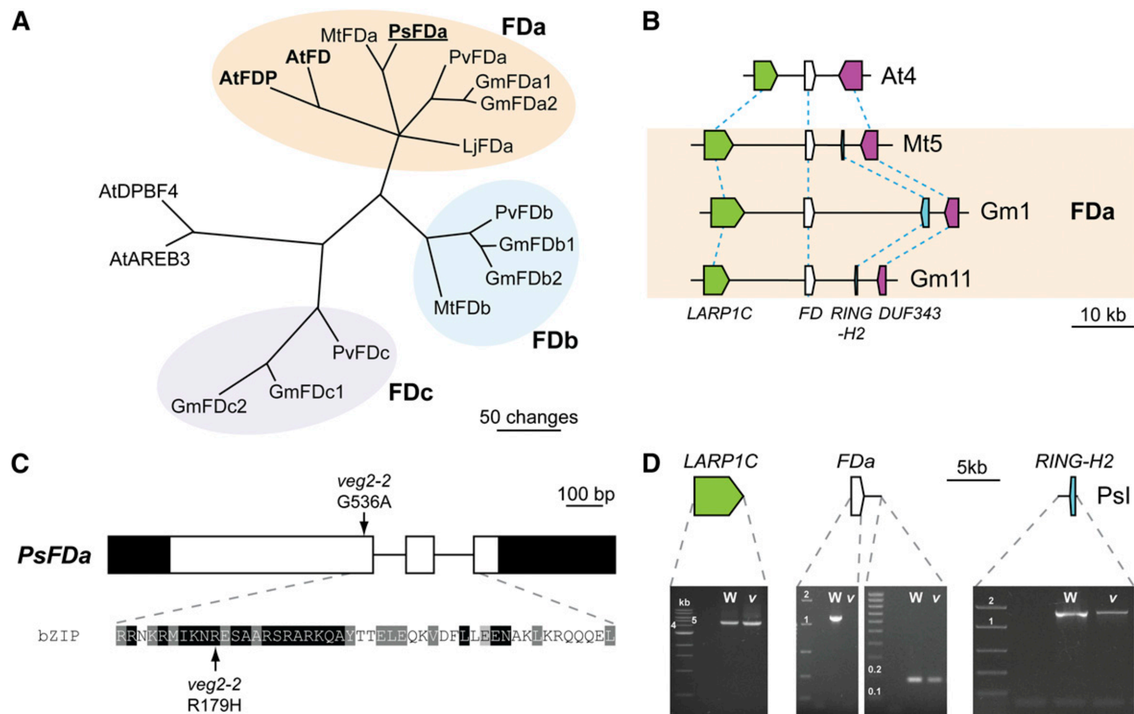

Figure 4. The *VEG2* Locus Corresponds to *FDa.*

(A) Phylogram of the legume FD family. Branches with bootstrap values <55% obtained from 10,000 trees have been collapsed. Two related group A bZIP transcription factors from Arabidopsis, DPBF4 and AREB3, are included as an outgroup. Alternative names for previously identified soybean proteins GmFDL02 (GmFDb1), GmFDL04 (GmFDc1), and GmFDL0602 (GmFDc2) are adopted to better reflect wider phylogenetic relationships. The analysis is based on the sequence alignment shown in Supplemental Data Set 1. Sequence details are given in Supplemental Table 2. Ps, *Pisum sativum*; At, *Arabidopsis thaliana*; Mt, *Medicago truncatula*; Lj, *Lotus japonicus*; Gm, *Glycine max*; Pv, *Phaseolus vulgaris*.

(B) Microsynteny between genomic regions containing Arabidopsis *FD*, legume *FDa* genes, and flanking genes. Genes are represented as boxes with point showing putative direction of transcription on black lines representing regions of the genome with chromosome number indicated. Between species, corresponding genes are connected by dashed lines. Microsynteny for legume *FDb* and *FDc* genes is shown in Supplemental Figure 8. Gene details are given in Supplemental Table 4.

(C) Diagram of the pea *FDa* gene showing nature and location of the SNP in *veg2-2*, which affects a conserved amino acid within the functional bZIP domain. Exons are shown as boxes, with coding sequence in white and untranslated regions in black. Shading levels in bZIP domain sequence indicate degree of conservation (black = 100%, dark gray = 80%, and light gray = 60%) from alignment with other FD proteins shown in Supplemental Figure 9. Nucleotide numbering begins at the start of the coding sequence.

(D) Representative PCR results for full-length coding sequence for *FDa*, its putative 5′ and 3′ flanking genes, and a region immediately downstream of *FDa* from the wild type (Kaliski) and *veg2-1* mutant genomic DNA template. For each gel, lanes containing a DNA ladder and PCR product for a no template negative control, wild-type (W) positive control, and *veg2-1* (*v*) mutant gDNA are shown (from left to right). Band size (kb) is indicated for relevant ladder bands. Above each gel, the genomic regions isolated are shown diagrammatically, as for **(B)**. Regions between gene diagrams are not drawn to scale.

same arginine-to-histidine substitution was previously reported for the maize *FD* homolog *DLF1* in the inflorescence mutant *dlf1-N2461A* (Muszynski et al., 2006). Results from 3D modeling indicated that the arginine at this position comes into direct contact with the phosphate groups on target DNA and conversion to a histidine results in distortion of the DNA backbone, which reduces binding strength (Muszynski et al., 2006). The R179H amino acid substitution in the pea *veg2-2* mutant is likely to reduce FDa function in a similar manner.

Attempts to amplify *FDa* by PCR from *veg2-1* genomic DNA were unsuccessful, whether using primers within or spanning the *FDa* coding sequence (Figure 4D), and this failure to amplify *FDa* clearly distinguished all *veg2-1* mutant plants (n = 37) in a segregating population (n = 210; data not shown). The simplest interpretation of these findings is that a deletion encompassing *FDa* has occurred in this mutant. As the pea genome sequence is not yet available, we again made use of microsynteny to investigate the extent of this apparent deletion. Figure 4B shows that the two genes flanking *FDa* in *M. truncatula*, *LARP1C* and a *RING-H2* gene, also have conserved positions flanking *FDa* in soybean, and in view of the close relationship of pea to *M. truncatula*, we considered it likely that this arrangement was also preserved in pea. The pea homologs of these genes were isolated, mapped, and found to be closely linked to each other and to *FDa*, as expected (Supplemental Figure 6), and full-length coding sequence for both genes was found to be intact in the *veg2-1* mutant (Figure 4D). In

addition, a fragment 1.4 kb downstream of the *FDa* stop codon was found to be present in *veg2-1*, revealing that the 3′ boundary for the *veg2-1* deletion is close to *FDa* coding sequence (Figure 4D). Further attempts to define the precise boundaries of the deletion by isolating the region between *LARP1C* and this fragment in the wild type and *veg2-1* mutant were unsuccessful. However, as both the flanking genes predicted by microsynteny were found to be intact in *veg2-1*, our results suggest that this mutant contains a deletion restricted to *FDa*. Therefore, based on the evidence of distinct functionally significant mutations that specifically affect *FDa* in both of the *veg2* mutants, and the correlation between the molecular nature of the mutations and the severity of the respective mutant phenotypes, we conclude that the *VEG2* locus corresponds to *FDa* and subsequently refer to *FDa* as the *VEG2* gene.

VEG2 Is Expressed in the Apex throughout Development

In view of observations that *VEG2* is important for multiple stages of inflorescence development, it was of interest to investigate the developmental and spatial pattern of *VEG2* expression. We first examined the expression of *VEG2* by quantitative RT-PCR (qRT-PCR) in shoot apex and leaf tissues of wild-type plants throughout development from seedling to flowering adult plant in LD. Figure 5A shows that *VEG2* was not significantly expressed in expanded leaves but was expressed in the shoot apex throughout development, where *VEG2* transcript levels increased during early vegetative growth and showed a further increase during later floral development.

The *VEG2* expression pattern in the wild-type apex was next investigated in more detail by in situ hybridization during the vegetative phase (Figure 5F), the V/I_1 transition (Figure 5G), and early flowering stages (Figures 5H to 5J). Apical samples from the *veg2-1* mutant were included as negative controls for the *VEG2* in situ probe (Figure 5B). Expression patterns for *VEG1* (Figure 5C) and *PIM* (Figures 5D and 5E) were determined on serial sections of the same apices used for *VEG2*, in order to identify I_2 and floral meristem boundaries, respectively (Taylor et al., 2002; Berbel et al., 2012). Consistent with previous reports, expression of *VEG1* was observed in I_2 meristems, and *PIM* expression was observed in floral meristems and floral primordia, in the petal region of the petal/stamen common primordia, and in the sepals (Figures 5C to 5E; Taylor et al., 2002; Berbel et al., 2012). *VEG2* was expressed in the vegetative SAM, axillary meristems, the I_1 meristem, I_2 meristems, vasculature, and tips of leaf primordia (Figures 5F to 5J). Expression was also seen in floral meristems during early development (Figure 5I) but was restricted to floral vasculature during later stages of development (Figure 5J). This expression pattern is consistent with roles for *VEG2* during the V/I_1 transition, in specification and maintenance of I_2 meristem identity, and in specification of floral meristem identity.

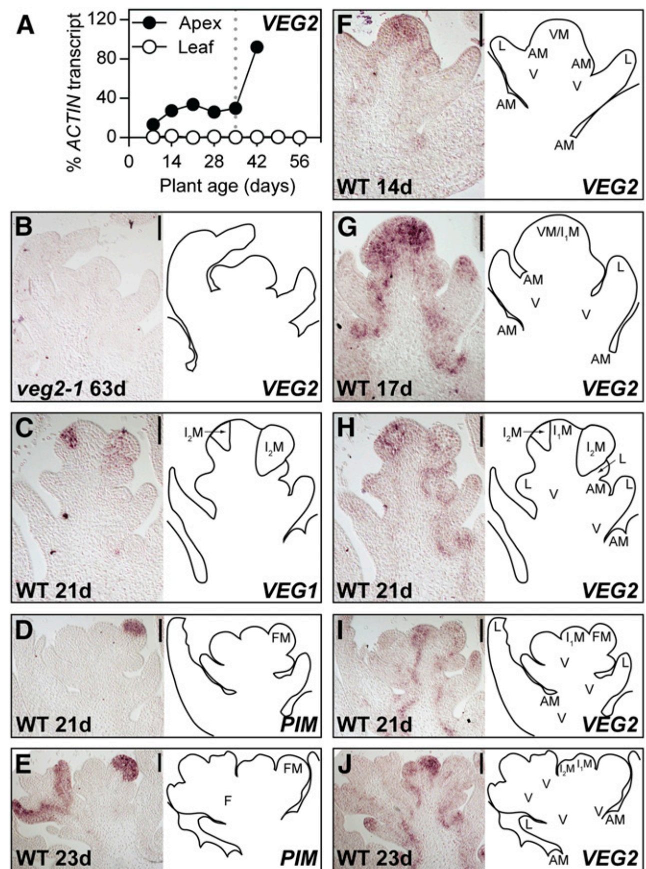

Figure 5. *VEG2* Is Expressed in the Apex throughout Development.

(A) Expression of *VEG2* in dissected shoot apices and the uppermost fully expanded leaf of wild-type (NGB5839) plants throughout development. Relative transcript levels were determined by qRT-PCR, normalized to the transcript level of *ACTIN*, and represent mean ± SE for *n* = 2 biological replicates, each consisting of pooled material from two plants grown in LD (24 light). Developing floral buds were first macroscopically visible in the wild-type apex 35 d after sowing (gray line).

(B) to **(J)** In situ hybridization results for *VEG2* and meristem marker genes.

(B) *VEG2* expression in the shoot apex of the *veg2-1* deletion mutant, as a negative control for the *VEG2* probe.

(C) *VEG1* expression domain, as a marker for secondary inflorescence (I_2) meristems.

(D) and **(E)** *PIM* expression domain, as a marker for floral meristems.

(F) to **(J)** *FDa* expression in the vegetative apex **(F)**, the apex at the approximate time of the transition from vegetative to primary inflorescence (I_1) meristem identity **(G)**, the I_1 apex during early I_2 and floral development (**[H]** and **[I]**), and the I_1 apex during development of floral primordia **(J)**.

Each pair, **(C)** and **(H)**, **(D)** and **(I)**, and **(E)** and **(J)**, represents serial sections from the same apex. Shoot apices shown in photographs in **(C)** to **(J)** are from wild-type (NGB5839) plants grown in LD (16 light/8 dark). For each sample, plant age in days after sowing (d) is indicated. Regions of expression are indicated by abbreviations on diagrams as follows: axillary meristem (AM), floral meristem (FM), developing flower (F), leaf primordia (L), primary inflorescence meristem (I_1M), secondary inflorescence meristem (I_2M), vasculature (V), and vegetative shoot apical meristem (VM).

Flowering and Meristem Identity Genes Are Misregulated in the *veg2* Mutants

To identify possible regulatory targets of *VEG2*, we next examined the expression of floral integrator and meristem identity genes in

the *veg2* mutants, including members of the *FT/TFL1* and MADS box gene families and the *LFY* ortholog *UNIFOLIATA* (*UNI*), under LD conditions. Gene expression was investigated in the same experiments described above for *DET* expression, first in a detailed time course in *veg2-2* and then in both *veg2* mutants at specific time points chosen to coincide with the appearance of floral buds in wild-type and *veg2-2* plants (45 and 74 d after sowing, respectively).

FTa1 and *FTc* are significantly expressed in wild-type apical tissue and are upregulated after commitment to flowering (Hecht et al., 2011; Figure 6A). A comparison of the two time points in Figure 6B suggests that upregulation of *FTa1* was delayed in both *veg2* mutants; more specifically an ~3-week delay was apparent in *veg2-2* in the detailed time course (Figure 6A). *FTc* expression was reduced by 4-fold at both time points in *veg2-1* and showed delayed induction, by ~1 week, in *veg2-2* (Figure 6). Also, in addition to *DET*, which exhibits delayed upregulation in both *veg2* mutants (Figures 2D and 2E), a second pea *TFL1* homolog, *LF* (*TFL1c*), was also misregulated, but in an opposite manner. This gene, which inhibits flowering (Foucher et al., 2003), was expressed up to 7-fold higher in the *veg2* mutants relative to wild-type levels (Figure 6B).

In Arabidopsis, the floral integrator and floral identity gene *LFY* defines an FT/FD-independent pathway for *AP1* upregulation (Ruiz-García et al., 1997; Abe et al., 2005; Wigge et al., 2005). In wild-type pea, the *LFY* ortholog *UNI* is expressed at a low level in the apex during early vegetative development and upregulated at the time of flowering, when *FTa1* and *FTc* are induced (Hecht et al., 2011; Figure 6A). This upregulation was delayed by ~4 weeks in *veg2-2* and *UNI* expression remained at a low level in *veg2-1*, more than 3-fold lower than wild-type levels at day 45 (Figure 6), indicating that *UNI* is downstream of *VEG2* in pea.

Several MADS box genes were also misexpressed in the *veg2* mutants. The I_2 identity gene *VEG1* and the floral identity gene *PIM* have important roles in specifying meristem identity, and consistent with previous reports, these genes were upregulated in the wild-type apex immediately prior to floral development, at a similar time to *FTa1* and *FTc* (Hecht et al., 2011; Berbel et al., 2012; Figure 6). In the *veg2-1* mutant, *VEG1* and *PIM* were not expressed, and in *veg2-2*, these genes showed an ~4 week delay in induction, roughly corresponding to the delay in flowering time (Figure 6). A similar pattern was also seen for expression of floral organ identity genes *AP3* and *SEPALATA1* (*SEP1*), consistent with the absence of flowers in *veg2-1* and delayed occurrence of floral development in *veg2-2* (Figure 6).

FDa/VEG2 Interacts with All Pea FT Proteins

Studies in diverse species have shown that the physical interaction of FD and FT proteins is widely conserved and functionally

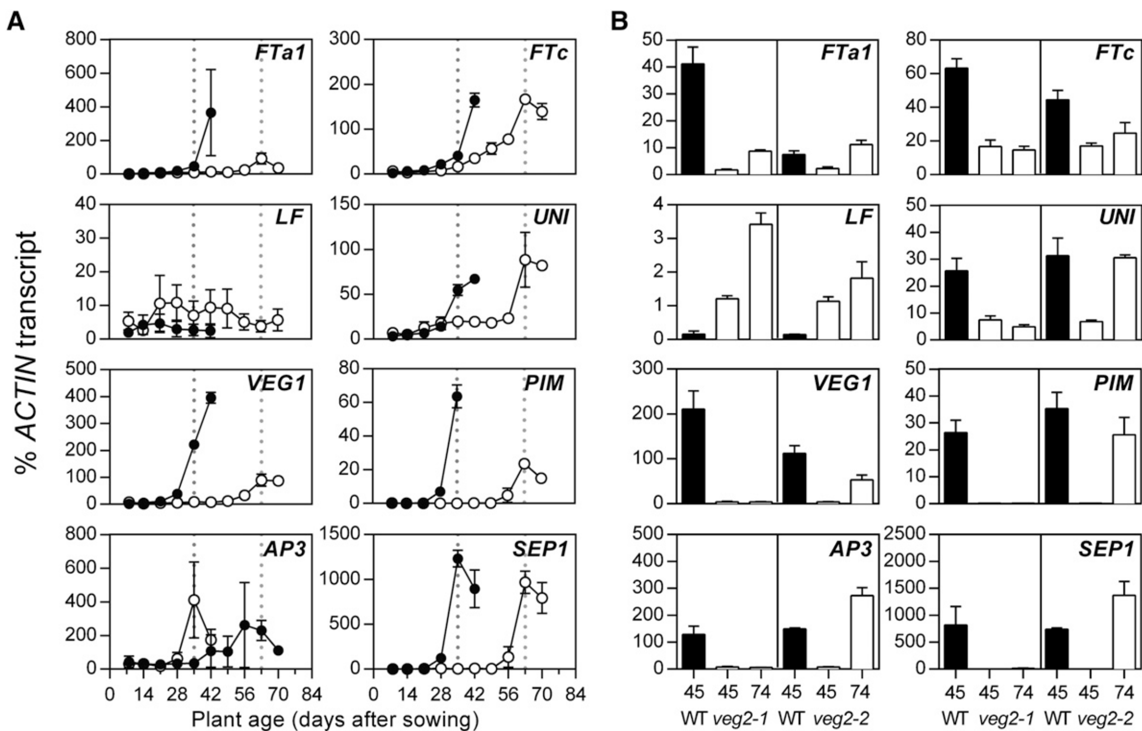

Figure 6. Flowering and Meristem Identity Genes Are Misregulated in the *veg2* Mutants.

Gene expression in dissected shoot apices at weekly time points throughout development in wild-type (NGB5839; closed circles) and *veg2-2* (open circles) **(A)** and specific time points in both *veg2* mutants and associated wild-type lines (wild-type siblings of *veg2-1* and NGB5839, respectively) **(B)**. In **(A)**, developing floral buds were first macroscopically visible in the wild type 35 d after sowing and in *veg2-2* 63 d after sowing (broken lines) in LD (24 light). In **(B)**, time points correspond to early flowering stages in the wild type 45 d after sowing and *veg2-2* plants 74 d after sowing in LD (18 light/6 dark). **(A)** and **(B)** show results from the same two experiments shown in Figures 2D and 2E, respectively. Mean values ± SE are shown for n = 2 to 3 biological replicates.

significant (Wigge et al., 2005; Taoka et al., 2011; Tsuji et al., 2013). However, in maize (*Zea mays*), where expansion of the *FT* family has resulted in functional divergence between family members, there is evidence that these FT proteins differ in their ability to interact with an FD homolog (Danilevskaya et al., 2008; Meng et al., 2011). Differences in expression pattern, differing effects in transgenic Arabidopsis, and inferences from the *gigas* phenotype all indicate a divergence of function within the pea *FT* family (Hecht et al., 2011), which could in part be determined by differences in interaction with VEG2/FDa.

To examine whether this was indeed the case, we tested the interactions of VEG2 with the five pea FT proteins using bimolecular fluorescence complementation analysis in *Nicotiana benthamiana* leaves. For all five combinations, reconstitution of yellow fluorescent protein (YFP) fluorescence was observed in the nuclei of *N. benthamiana* leaf epidermal cells (Figure 7), at levels clearly above background (Supplemental Figure 10), indicating that VEG2 can interact with all five pea FT proteins in planta.

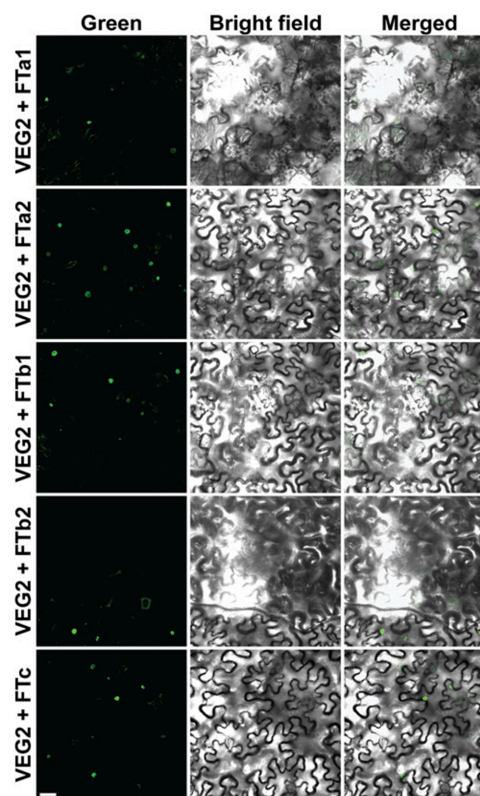

Figure 7. VEG2/FDa Can Interact with Each Pea FT Protein in Planta.

For each interaction, VEG2 fused to the N-terminal half of YFP (YFN) was coexpressed separately with the FT protein fused to the C-terminal half of YFP (YFC). Photographs from left to right comprise the green channel image showing fluorescence of YFP, the bright-field image and the merged YFP fluorescence, and bright-field images. Positive and negative controls are shown in Supplemental Figure 10. Bars = 40 μm.

DISCUSSION

The pea inflorescence is typical of many legumes and is distinguished from the simple Arabidopsis inflorescence by an additional level of branching, with a so-called secondary inflorescence (I_2) that displaces flowers from the main inflorescence stem (Figure 1). Three pea loci, *GIGAS*, *VEG1*, and *VEG2*, affect the formation of I_2s and thus have the potential to provide insight into the genetic mechanisms that direct compound inflorescence development (Benlloch et al., 2007). *GIGAS* and *VEG1* have been recently characterized as homologs of *FT* and *AGL79*, respectively (Hecht et al., 2011; Berbel et al., 2012). Here, we characterized the third of these loci, *VEG2*, as a pea homolog of the bZIP transcription factor *FD* and investigated its roles and interactions in the initiation of flowering and throughout inflorescence development.

VEG2 Participates in the Initiation of Flowering

From a developmental perspective, the initiation of flowering in pea is closely associated with the acquisition of I_1 identity by the SAM, which is marked by *DET/TFL1a* expression (Berbel et al., 2012). In *veg2* mutants, the induction of *DET* is delayed relative to the wild type and the other nonflowering mutants, *gigas* and *veg1* (Figures 2D and 2E; Supplemental Figure 3). This indicates that *VEG2* has an important role in promoting the V/I_1 transition in wild-type plants under LD conditions, whereas *GIGAS/FTa1* and *VEG1* do not, despite the apparent similarity of their mutant phenotypes. However, the eventual induction of the I_1 marker gene *DET* in the *veg2-1* deletion mutant (Figure 2E) does suggest that at least one *VEG2*-independent pathway also promotes the V/I_1 transition in pea.

The majority of work on *FD* genes in other species has focused on their participation in florigen signaling. Several studies have now shown that FD proteins physically associate with FT proteins and are essential for their flower-promoting function, providing a crucial link between FT proteins and their transcriptional targets (Abe et al., 2005; Wigge et al., 2005; Taoka et al., 2011). The FD/FT interaction has been examined in most detail in rice (*Oryza sativa*), where it has been shown that the OsFD1 protein does not bind directly to the FT protein Hd3a, but that their interaction is mediated by 14-3-3 proteins (Taoka et al., 2011; Tsuji et al., 2013). These FT/14-3-3/FD complexes have been referred to as florigen activation complexes (FACs), and it is likely that VEG2 also acts as part of one or more FACs in pea. Pea has five *FT* genes, and analysis of expression patterns, mutant phenotypes, and activity in transgenic Arabidopsis suggest that these genes may have distinct roles in the flowering process (Hecht et al., 2011). Our results indicate that VEG2 can interact with each of the five pea FT homologs in planta (Figure 7), which suggests that participation in FACs with VEG2 may be important for the function of all pea FT proteins, but appears to rule out differential VEG2 binding as an explanation for differences in their function. Future investigations should instead explore the recent hypothesis that FT functional specificity may in fact derive from interactions with other proteins via a domain distinct from residues required for FAC binding (Ho and Weigel, 2014).

Among the five pea *FT* genes, only three (*FTa1*, *FTb2*, and *FTc*) show clear developmental regulation, consistent with roles in initiation of flowering and/or inflorescence development (Hecht et al., 2011). Because the V/I_1 transition is the first stage

of inflorescence development, it is likely that the role of VEG2 in this process involves interaction with the FT protein/s that act as florigens and move from leaf to apex following perception of appropriate environmental signals. Grafting experiments with *gigas* mutants suggest that GIGAS/FTa1 may function as one such mobile signal (Beveridge and Murfet, 1996; Hecht et al., 2011). However, the fact that induction of the I_1 marker *DET* is not affected by the *gigas* mutation shows that the FAC involving FTa1 is probably not important for initiating the V/I_1 transition. This is also consistent with the fact that induction of *FTa1* in leaves is delayed relative to floral commitment and with the fact that the *gigas* phenotype indicates a role later in inflorescence development, promoting I_2 specification rather than the V/I_1 transition. A second *FT* gene, *FTb2*, is also a strong candidate for a florigen signal, as it is induced in leaves within the time frame of the physiological commitment to flowering in LD and is severely misregulated in photoperiod response mutants (Hecht et al., 2011). Furthermore, *FTb2* is expressed normally in the *gigas* mutant in LD (Hecht et al., 2011), which could account for the fact that the V/I_1 transition is not affected in the *gigas* mutant under these conditions. Functional analysis of *FTb2*, and in particular whether it regulates expression of *DET*, will be important to clarify its involvement in the V/I_1 transition.

VEG2 Is Essential for Secondary Inflorescence Development

We recently showed that expression of the MADS box gene *VEG1* is crucial for the formation of the I_2 and proposed that I_2 meristem identity is specified by *VEG1* (Berbel et al., 2012). The nonflowering *veg2-1* and *gigas* mutants are unable to form I_2 structures and do not show *VEG1* expression (Berbel et al., 2012; Figure 6), which indicates that *VEG2/FDa* and *GIGAS/FTa1* are both required for induction of *VEG1* under LD conditions. In addition, our data indicate that the VEG2 and FTa1 proteins can interact (Figure 7), implying that they participate in a FAC that acts to initiate *VEG1* expression and specify I_2 meristem identity. The fact that the abnormal I_2s in the weaker *veg2-2* mutant are indeterminate and revert to I_1 identity (Figure 3) shows that *VEG2/FDa* also has a role not only in initial specification of I_2 identity, but also in maintenance of this identity. The incomplete specification of I_2 identity in *veg2-2* is accompanied by a reduction in *VEG1* expression levels relative to the wild type (Figure 6), which is consistent with the idea that *VEG1* expression is a critical limiting factor in I_2 development.

We previously proposed a model for inflorescence development in pea, which elaborates on the simple *TFL1/AP1* negative feedback loop described in Arabidopsis (Ratcliffe et al., 1999; Kaufmann et al., 2010). In this model, *DET* prevents upregulation of *VEG1* and *PIM* in the I_1 meristem, and *VEG1* prevents upregulation of *DET* in the I_2 meristem, allowing expression of *PIM* in axillary floral meristems (Berbel et al., 2012). The incomplete and transient specification of I_2 identity in the hypomorphic *veg2-2* mutant illustrates how disruption of a regulatory loop can destabilize a sharp developmental transition (Sablowski, 2007). This role for *VEG2/FDa* in the maintenance of I_2 identity is comparable to the role recently described for *FT* in Arabidopsis, in stabilizing inflorescence meristem identity after flowering to prevent floral reversion (Liu et al., 2014; Müller-Xing et al., 2014).

One significant point of contrast between *VEG2* and *FD* genes in other species lies in the severity of its null mutant phenotype. Whereas the *veg2-1* mutant is completely unable to flower, *FD* mutants in both Arabidopsis and maize are merely late-flowering (Koornneef et al., 1991; Abe et al., 2005; Wigge et al., 2005; Muszynski et al., 2006). Even when FD/FT function is completely absent in Arabidopsis, in *fd fdp* or *ft tsf* double mutants, flowering will still occur, albeit considerably later than in any single mutant (Jang et al., 2009; Jaeger et al., 2013). However, a nonflowering phenotype is seen in Arabidopsis when *fd* or *ft* mutations are combined with *lfy*, indicating that *LFY* acts in parallel with *FD* and *FT* genes to upregulate *AP1* for specification of floral meristems (Ruiz-García et al., 1997; Abe et al., 2005; Wigge et al., 2005). In pea, the *LFY* ortholog *UNI* is expressed at a low level in vegetative seedlings where it has a role in leaf development (Hofer et al., 1997), but is upregulated at the time of flowering, and this upregulation is dependent on *FTa1* (Hecht et al., 2011). Our results show that the upregulation of *UNI* is also dependent on *VEG2* (Figure 6), which suggests that *UNI* is acting downstream of *VEG2* and *FTa1* in pea, and not in parallel, as is seen for *LFY* in other systems.

A second factor that may contribute to the severity of the *VEG2* null phenotype is the existence of I_2 specification as an intermediate step in pea inflorescence development and the essential role of *VEG1* in this process. All three nonflowering mutants (*gigas*, *veg1*, and *veg2-1*) show a correlation between the absence of *VEG1* expression and failure to express *PIM* (Figure 6B; Hecht et al., 2011; Berbel et al., 2012), suggesting that *VEG1* expression is an absolute requirement for *PIM* expression to occur in secondary inflorescences in the presence of functional *DET*. The fact that *PIM* is expressed in the *veg1 det* double mutant (Berbel et al., 2012) suggests that lack of *PIM* expression in *veg1* may reflect a strong suppression of *PIM* by *DET* that is relieved through repression of *DET* by *VEG1*. The delay in *PIM* induction observed in the *veg2-2* mutant (Figure 6) suggests that *VEG2* promotes *PIM* expression, either directly, or indirectly through repression of the floral repressor *LF/TFL1c*.

VEG2 Activity in Floral Meristems Implies a Role for *FT* Genes in Flower Development

The *veg2-2* mutant phenotype also reveals that *VEG2* has a role in correct specification of floral identity, especially for sepal and petal whorls (Figure 3L). The observed acropetal decrease in severity of floral defects (Figure 3M; Supplemental Figure 4) indicates that *VEG2* is especially important for correct floral development at early reproductive nodes, but this importance decreases with plant age. This could be due either to slow accumulation of downstream targets through partial *VEG2* function in the *veg2-2* mutant or their activation via an alternative, age-related pathway. The floral abnormalities seen in *veg2-2* are similar to those seen in mutants for *PIM*, which is misregulated in the *veg2-2* mutant. Like *veg2-2*, *pim* mutants exhibit replacement of sepals with leafy bracts, second and third whorl organs are missing or mosaic, and severity of floral morphology defects decreases acropetally (Singer et al., 1999; Taylor et al., 2002). However, the severity of the *pim veg2-2* double mutant phenotype indicates that *VEG2* probably has other targets in addition to *PIM*, most likely other MADS box genes, such as

Figure 8. A Model for the Roles and Interactions of *VEG2* during Pea Inflorescence Development.

This model summarizes the main hypotheses derived from the major results of this study and previous studies. We propose that FTb2, the best candidate for the pea florigen signal, travels from the leaf to the shoot apex under LD and interacts within a FAC with VEG2/FDa in the apex to promote primary inflorescence meristem (I_1M) identity, through upregulation of *DET*, *GIGAS/FTa1*, and *FTc* and repression of the floral repressor *LF*. *FTa1* is expressed in the leaf, encodes a graft-transmissible floral stimulus, and is also expressed in the apex. We infer that FTa1 protein acts in a FAC with VEG2 in the apex to promote expression of *FTc* and induce *VEG1* for specification of secondary inflorescence meristem (I_2M) identity. Repressive interactions between *DET*, *VEG1*, and *PIM* are based on a previous model (Berbel et al., 2012). Proteins and protein complexes are shown as ovals, and genes are shown in italics. Unbroken lines indicate inferred roles for genes/proteins as promoting (arrows) or repressing (blunt ends) expression of other genes, either directly or indirectly. Broken lines indicate movement of proteins or translation of genes into proteins. Colored zones indicate specific meristems boundaries. Genes/proteins shown outside colored meristem zones are not intended to represent spatial patterns of expression within the apex.

AP3 and *SEP1*, which are also misregulated in the *veg2-2* mutant (Figure 6). MADS box transcription factors are known to act in a combinatorial fashion to guide different stages of the flower initiation and development process (Smaczniak et al., 2012), and the persistent effects of *VEG2* might reflect the participation of a limited number of direct *VEG2* targets in MADS tetramers throughout reproductive development. Alternatively, *VEG2* may be required for direct induction of flower-specific MADS genes, which would also imply an extended postflowering role for *FT* genes and associated FACs. Our results here and in the previous study of Hecht et al. (2011) show that expression of *FTa1* and *FTc* genes is induced in shoot apical tissue only after the initial commitment to flowering and continues well beyond this transition, consistent with a role in later reproductive development.

VEG2 and the Integration of *FT/TFL1* Signaling

In a previous study, we presented evidence for potential cross-regulation among *FT* genes, specifically, for the positive regulation of *FTc* at the shoot apex by *FTa1* and *FTb2* (Hecht et al., 2011). Results from this study show that *FTc* is also misregulated in the *veg2* mutants (Figure 6), providing support for the conclusion that one or more other *FT* genes may be required for full activation of *FTc*. In addition, *FTa1* expression in the shoot apex is also altered in the *veg2* mutants (Figure 6), implying that it too is dependent on one or more *FT* genes acting together with *VEG2*. This is consistent with a scenario in which both *FTa1* and *FTc* are transcriptional targets of FT proteins that arrive to the shoot apex, a conclusion also supported by the fact that induction of *FTa1* and *FTc* in the apex occurs only after the plant becomes committed to flower.

One interpretation is that these genes may act as functional integrators of mobile FT signals. However, the induction of *DET*, which marks the V/I_1 transition, occurs with very similar timing to that of *FTa1* and *FTc* in the shoot apex, with no evidence of the delay that might be expected if it was dependent on the expression of these genes. *GIGAS/FTa1* is clearly required for *VEG1* induction and I_2 specification, but the role of *FTc* is less clear. *FTc* is still expressed in *gigas*, albeit at a lower level than the wild type, and the fact that *gigas* plants do not flower under LD implies that *FTc* alone cannot substitute for *FTa1* in the initiation of I_2 formation under these conditions. One explanation is that *FTc* may play a subsidiary role to *FTa1*, reinforcing its expression and/or activity and ensuring a clear induction of *VEG1* and a sharp developmental transition from vegetative branch to I_2.

The observation that *VEG2* regulates *DET* expression provides intriguing evidence for transcriptional regulation of a *TFL1* gene by an *FD* gene. Given the mechanism for *FD* action, this also implies the involvement of *FT* family members, and some evidence for this does exist. In strawberry (*Fragaria vesca*), the *FT* gene *FT1* has been shown to activate *TFL1* expression indirectly via *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1*, to repress flowering and allow production of vegetative shoots under LD conditions in spring and summer (Mouhu et al., 2013). Similarly in Arabidopsis, *TFL1* is strongly upregulated in the SAM at the onset of flowering and is expressed in proportion to *FT* (Jaeger et al., 2013). Furthermore, a computational model designed to simulate flowering in Arabidopsis required the inclusion of a term for activation of *TFL1* by FT/FD proteins to correctly model the maintenance of SAM indeterminacy during flowering (Jaeger et al., 2013). Collectively, these findings suggest that direct or indirect transcriptional regulation of *TFL1* homologs by *FT* and *FD* homologs is also likely to occur more widely, and the functional significance of this and the mechanisms by which it is achieved will be of interest to determine.

In contrast to *DET*, the expression of the related *LF/TFL1c* gene was increased in both *veg2* mutants relative to the wild type (Figure 6). The tissue specificity of *LF* expression is not yet known, but the fact that *lf* mutants are early flowering without any other apparent defects can be interpreted to indicate that *LF* acts to prevent the acquisition of I_2 identity in lateral meristems. In this respect, it is similar to *DET*, which also prevents the acquisition of I_2 identity, but in the SAM. The finding that *VEG2* regulates these two *TFL1* homologs in opposite ways (i.e., promoting induction of

DET and repressing expression of *LF*) is consistent with the meristem identity changes that occur during the transition to flowering, as *DET* expression is positively associated with inflorescence development, while *LF* expression is not. It also implies that one or more FT proteins may act via FACs to relieve suppression of I_2 identity by *LF* in lateral meristems. In other angiosperms, including eudicot and monocot species, FD proteins have been found to also interact with TFL1 proteins, in complexes that inhibit the transcription of floral target genes (Pnueli et al., 2001; Danilevskaya et al., 2010; Hanano and Goto, 2011). The possibility that VEG2/FDa may interact with DET/TFL1a and/or LF/TFL1c in a similar manner in pea remains to be investigated.

The observations and hypotheses resulting from this study are summarized in the model in Figure 8, which suggests that FTb2 arriving at the shoot apex in LD may form a FAC with VEG2 and regulate a number of other *FT/TFL1* genes in the SAM and lateral meristems. The collective action of these secondary FACs may then allow specific expression of *VEG1* and formation of I_2s in lateral meristems, where *LF* has been downregulated, and promote upregulation of *DET* in the SAM to maintain SAM indeterminacy. Future work to test these ideas should include a detailed analysis of expression dynamics of *FT* and *LF* genes within the SAM, an examination of the interactions between VEG2 and TFL1 proteins, and characterization of *FTb2* and *FTc* mutants.

Overall, the findings from this study have extended our previous work to make a significant contribution to understanding of how differences in inflorescence architecture are generated. They will also assist the investigation of this process in a range of other important legume crop species that share similar inflorescence architecture. Understanding the complex network of genes controlling inflorescence development may ultimately contribute to crop improvement through optimization of inflorescence architecture for efficient harvest and maximal yield.

METHODS

Plant Material and Growth Conditions

The origins of pea (*Pisum sativum*) *veg2-1*, *veg2-2*, *pim-2*, *gigas-2*, and *veg1* mutants have been described previously (Gottschalk, 1979; Murfet and Reid, 1993; Taylor et al., 2002; Hecht et al., 2011). Molecular characterization of the *veg2-1* allele compared the mutant line in the original cv Kaliski background with cv Kaliski. All other experiments used mutant lines derived from multiple backcrosses in the dwarf NGB5839 background, as previously described (Hecht et al., 2007). In the case of nonflowering *veg1* and *veg2-1* mutants, wild-type siblings were used as controls. Plants for the qRT-PCR experiments shown in Figures 2D, 5A and 6A were grown in growth cabinets at 20°C, and plants for all other experiments were grown in the Phytotron. Growth media, light sources, Phytotron conditions, and grafting protocols have been described previously (Weller et al., 1997; Hecht et al., 2007). For branching data shown in Figure 2C, plants were measured 97 d after sowing and measurements include all vegetative laterals 5 mm or longer in length.

Gene Isolation

Partial length Ps *FDa* was isolated from a cDNA library screen of 1,000,000 clones from a 5′ Stretch Plus λgt11 cDNA library (Clontech) from pea apical buds (Lester et al., 1997) using partial Mt *FDa* as a probe. Partial Ps *FDa* sequence was extended using rapid amplification of cDNA ends (SMART RACE cDNA amplification kit; Clontech), genome walking (GenomeWalker Universal kit; Clontech), and standard PCR techniques to obtain full-length coding sequence. Putative *FDa* flanking genes and molecular marker genes for mapping of *FDa/VEG2* were isolated using primers designed from either pea sequence, where available, or conserved regions of *Medicago truncatula* orthologs. Primer details are given in Supplemental Table 3. PCR fragments were purified and sequenced directly or cloned in pGEM-T Easy (Promega) and then sequenced at the Australian Genome Research Facility (Brisbane, Australia) or Macrogen (Seoul, Korea).

Phylogenetic Analysis

FD genes from other species were identified by performing BLAST searches using *Arabidopsis thaliana* FD protein sequence as a query and identity was confirmed by reciprocal BLAST search against Arabidopsis at TAIR (www.arabidopsis.org) and preliminary phylogenetic analysis (data not shown). For full sequence details, including source, see Supplemental Table 2. For each alignment (Supplemental Data Sets 1 and 2), full-length amino acid sequence was aligned using ClustalX (Thompson et al., 1997) and adjusted manually, where necessary, using GeneDoc (version 2.7.000; Nicholas and Nicholas, 1997; http://www.psc.edu/biomed/genedoc). Using these alignments, distance-based methods were used for phylogenetic analyses in PAUP* 4.0b10 (http://paup.csit.fsu.edu/).

Gene Expression Studies

For qRT-PCR, harvested tissue for each sample consisted of both leaflets from the uppermost fully expanded leaf or dissected apical buds (~2 mm) from two plants. Samples were frozen in liquid nitrogen and total RNA extracted using the SV Total RNA isolation system (Promega). RNA concentrations were determined using a NanoDrop 8000 (Thermo Scientific). Reverse transcription was conducted in 20 μL with 1 μg total RNA using the Tetro cDNA synthesis kit (Bioline) according to the manufacturer's instructions. RT-negative (no enzyme) controls were performed to monitor for contamination with genomic DNA. First-strand cDNA was diluted five times, and 2 μL was used in each real-time PCR. Reactions using SYBR green chemistry (Sensimix; Bioline) were set up with a CAS-1200N robotic liquid handling system (Corbett Research) and run for 50 cycles in a Rotor-Gene RG3000 (Corbett Research). Two technical replicates and at least two biological replicates were performed for each sample. All primer details are given in Supplemental Table 3.

RNA in situ hybridization with digoxigenin-labeled probes was performed as previously described (Ferrándiz et al., 2000). Probes used for *VEG1* and *PIM* have been described previously (Berbel et al., 2001; Berbel et al., 2012). Primer details for the *VEG2/FDa* probe are given in Supplemental Table 3.

Bimolecular Fluorescence Complementation Assay

Full-length coding sequences of *VEG2/FDa*, *FTa1*, *FTa2*, *FTb1*, *FTb2*, and *FTc* were amplified from NGB5839 cDNA, cloned in-frame into the pCR8/GW/TOPO entry vector (Invitrogen), and transferred by Gateway LR reaction (Invitrogen) into $pYFP^{N}43$ and $pYFP^{C}43$ destination vectors. Primer details are given in Supplemental Table 3. Constructs for the positive control interaction between Arabidopsis proteins AKIN10 and AKINβ2 (Supplemental Figure 10) have been described previously (Belda-Palazón et al., 2012). Constructs were introduced into *Agrobacterium tumefaciens* C58C1 (pGV2260) and used to infiltrate young fully expanded leaves of 4-week-old tobacco (*Nicotiana benthamiana*) plants as previously described (Scacchi et al., 2009). Leaves were examined after 3 to 4 d with a Leica TCS-SL confocal microscope and a confocal laser scanning imaging system.

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics (version 21), using a significance level of 0.05. Levene's test for homogeneity of variance was applied, and one-way ANOVA (with Tukey's HSD post-hoc test) or Welch's test for ANOVA (with Games-Howell post-hoc test) were conducted, as appropriate.

Accession Numbers

Please refer to Supplemental Table 2 for details of sequences used in phylogenetic analyses and to Supplemental Table 4 for details of *FD* and flanking genes in Arabidopsis and legume species. GenBank accession numbers for other pea genes are as follows: *AP3* (JN412098), *DET* (AY340579), *FTa1* (HQ538822), *FTa2* (HQ538823), *FTb1* (HQ538824), *FTb2* (HQ538825), *FTc* (HQ538826), *LARP1C* (JI919144, JI924790, and JR963915), *LF* (AY343326), *PIM* (AJ291298), *RING-H2* (KP739948), *SEP1* (AY884290), *UNI* (AF010190), *VEG1* (JN974184), and *VEG2/FDa* (KP739949 and KP739950).

Supplemental Data

Supplemental Figure 1. Phenotypes for key inflorescence mutants in pea.

Supplemental Figure 2. Branching in the *veg2* mutants.

Supplemental Figure 3. *DET* expression in nonflowering *gigas* and *veg1* mutants.

Supplemental Figure 4. Floral morphology in the *veg2-2* mutant.

Supplemental Figure 5. The *pim-2 veg2-2* double mutant phenotype.

Supplemental Figure 6. Comparative map for pea and *M. truncatula* showing relative locations of *VEG2/FDa* and surrounding genes.

Supplemental Figure 7. Phylogram of the angiosperm FD family.

Supplemental Figure 8. Microsynteny between genomic regions containing *FD* and flanking genes in Arabidopsis and legume species.

Supplemental Figure 9. Conserved nature of the amino acid altered by the *veg2-2* SNP.

Supplemental Figure 10. Positive and negative BiFC controls.

Supplemental Table 1. Mapping loci details.

Supplemental Table 2. Details of sequences for FD proteins and related bZIP transcription factors used for phylogenetic analyses and alignments.

Supplemental Table 3. Primer details.

Supplemental Table 4. Details for *FD* and flanking genes in Arabidopsis and legume species.

Supplemental Data Set 1. Alignment used for Figure 4A.

Supplemental Data Set 2. Alignment used for Supplemental Figure 7.

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AUTHOR CONTRIBUTIONS

F.C.S., J.L.W., V.H., F.M., and C.F. designed the research. F.C.S., A.B., and J.K.V.S. performed the research. F.C.S. and J.L.W. wrote the article.

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